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ATC ATC CGC AAC 1212
Ile Ile Arg Asn
404

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid (synthetic DNA)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CAACATGTCG TCAGTCATAT GTGTTTCCTG TGTGAAATT 39

What is claimed is:

1. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction; creatine+H_2O→sarcosine+urea

Optimum temperature: about 40–50° C.

Optimum pH: pH about 8.0–9.0

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5–10.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 3.5.

2. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction; creatine+H_2O→sarcosine+urea

Optimum temperature: about 40–50° C.

Optimum pH: pH about 8.0–9.0

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 4.5±1.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 3.5.

3. The creatine amidinohydrolase of claim **2**, which is obtained from *Escherchia coli* JM109 (pCRH273M2) (FERM BP-5375).

4. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction; creatine+H_2O→sarcosine+urea

Optimum temperature: about 40–50° C.

Optimum pH: pH about 8.0–9.0

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 6.5±1.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 3.5.

5. The creatine amidinohydrolase of claim **4**, which is obtained from *Escherchia coli* JM109 (pCRH273M1) (FERM BP-5374).

6. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction; creatine+H_2O→sarcosine+urea

Optimum temperature: about 40–50° C.

Optimum pH: pH about 8.0–9.0

K_m value for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 9.0±1.0 mM

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectric point: about 3.5.

7. The creatine amidinohydrolase of claim **6**, which is obtained from *Escherchia coli* JM109 (pCRH273M3) (FERM BP-5376).

8. A method for producing the creatine amidinohydrolase of claim **1**, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.

9. The method of claim **8**, wherein said microorganism is selected from the group consisting of *Escherichia coli* JM109 (pCRH273M1) (FERM BP-5374), *Escherichia coli* JM109 (pCRH273M2) (FERM BP-5375) and *Escherichia coli* JM109 (pCRH273M3) (FERM BP-5376).

10. A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim **1**, a sarcosine oxidase and a composition for the detection of hydrogen peroxide.

11. The reagent of claim **10**, in which the composition for the detection of hydrogen peroxide comprises an enzyme having a peroxidase activity, a chromophore and a buffer.

12. The reagent of claim **11**, in which the enzyme having the peroxidase activity is selected from the group consisting of peroxidase, haloperoxidase, bromoperoxidase, lactoperoxidase and myeloperoxidase.

13. The reagent of claim **11**, in which the chromophore comprises a hydrogen receptor and a coupler.

14. The reagent of claim **13**, in which the hydrogen receptor is 4-aminoantipyrine or a 3-methyl-2-benzothiazoline-hydrazine derivative.

15. The reagent of claim **13**, in which the coupler is an aniline derivative or a phenol derivative.

16. A method for determining creatine in a sample, which comprises measuring the absorbance of the pigment produced by the reaction of the reagent of claim **10** with the sample.

17. A reagent for determination of creatinine in a sample, comprising a creatinine amidohydrolase, the creatine amidinohydrolase of claim **1**, a sarcosine oxidase and a composition for the detection of hydrogen peroxide.

18. The reagent of claim **17**, in which the composition for the detection of hydrogen peroxide comprises an enzyme having a peroxidase activity, a chromophore and a buffer.

19. The reagent of claim **18**, in which the enzyme having the peroxidase activity is selected from the group consisting of peroxidase, haloperoxidase, bromoperoxidase, lactoperoxidase and myeloperoxidase.

20. The reagent of claim **18**, in which the chromophore comprises a hydrogen receptor and a coupler.

21. The reagent of claim **20**, in which the hydrogen receptor is 4-aminoantipyrine or a 3-methyl-2-benzothiazoline-hydrazine derivative.

22. The reagent of claim **20**, in which the coupler is an aniline derivative or a phenol derivative.

23. A method for determining creatinine in a sample, which comprises measuring the absorbance of the pigment produced by the reaction of the reagent of claim **17** with the sample.

* * * * *

24. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM.

25. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation)

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM.

26. The creatine amidinohydrolase of claim 24, which is stable at pH 5-8 (40 °C, 18 h preservation).

27. The creatine amidinohydrolase of claim 24, which has the following physicochemical properties:

Optimum temperature: about 40-50 °C

Optimum pH: about 8.0-9.0.

28. The creatine amidinohydrolase of claim 24, which has a molecular weight of about 43,000 (SDS-PAGE).

29. The creatine amidinohydrolase of claim 25, which has the following physicochemical properties:

Optimum temperature: about 40-50 °C

Optimum pH: about 8.0-9.0.

30. The creatine amidinohydrolase of claim 25, which has a molecular weight of about 43,000 (SDS-PAGE).

31. The creatine amidinohydrolase of claim 26, which has the following physicochemical properties:

Optimum temperature: about 40-50 °C

Optimum pH: about 8.0-9.0.

32. The creatine amidinohydrolase of claim 26, which has a molecular weight of about 43,000 (SDS-PAGE).

33. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM

Optimum temperature: about 40-50 °C

Optimum pH: pH about 8.0-9.0

Molecular weight: about 43,000 (SDS-PAGE).

34. The creatine amidinohydrolase of claim 33, which has the following physicochemical properties:

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation)

Heat stability: not more than about 50 °C (pH 7.5, 30 min).

35. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation)

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 4.5±1.0 mM.

Optimum temperature: about 40-50 °C

Optimum pH: pH about 8.0-9.0

Molecular weight: about 43,000 (SDS-PAGE).

36. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation).

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 6.5±1.0 mM.

Optimum temperature: about 40-50 °C

Optimum pH: pH about 8.0-9.0

Molecular weight: about 43,000 (SDS-PAGE).

37. A creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Heat stability: not more than about 50 °C (pH 7.5, 30 min)

pH stability: being stable at pH 5-8 (40 °C, 18 h preservation).

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 9.0±1.0 mM.

Optimum temperature: about 40-50 °C

Optimum pH: pH about 8.0-9.0

Molecular weight: about 43,000 (SDS-PAGE).

38. A method for producing the creatine amidinohydrolase of claim 24, comprising culturing a microorganism producing said creatine amidinohydrolase in a nutrient medium and recovering said creatine amidinohydrolase from the resulting culture.

39. A reagent for determination of creatine in a sample, comprising the creatine amidinohydrolase of claim 24, a sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

40. A method for determining creatine in a sample, which comprises measuring an absorbance of a pigment produced by the reaction of the reagent of claim 39 with the sample.

41. A reagent for determination of creatinine in a sample, comprising a creatinine amidohydrolase, the creatine amidinohydrolase of claim 24, sarcosine oxidase, and a composition for the detection of hydrogen peroxide.

42. A method for determining creatinine in a sample, which comprises measuring an absorbance of a pigment produced by the reaction of the reagent of claim 41 with the sample.